Occurrence of Toxic Bivalves in Association with *Gonyaulax* Planktons in Ise, Owase, and Ofunato Bays

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The occurrence of toxic bivalves was surveyed in several bays in association with *Gonyaulax* planktons. Various bivalves, such as short-necked clam and mussel, collected in April and May, 1976, in Ise Bay showed a maximum toxicity of 100 MU/g of whole soft body. The mussel collected in January and February, 1977, in Owase Bay exhibited a maximum toxicity of 70 MU/g of whole soft body. In the former case, a *Gonyaulax* bloom occurred almost in parallel with the appearance of toxic bivalves, indicating their close association. In the latter case, no bloom was observed.

On the other hand, as high a toxicity as 800 MU/g was encountered with the mid-gut gland of scallop collected in May, 1976, in Ofunato Bay where no visible *Gonyaulax* bloom has so far been observed. The toxicity values of the ovary and adductor muscle of scallop were 45 and 20 MU/g, respectively.

As previously reported¹⁾, the bloom due to a toxic dinoflagellate *Gonyaulax catenella* occurred in January and February, 1975, in Owase Bay, Mie Pref., resulting in the appearance of toxic shellfishes. This was the first case where a close association was indicated between the bloom and the toxic bivalves.

Since then, similar blooms occurred in not only Owase Bay but also some other bays, i.e., Ise Bay and Komatsushima Bay, Tokushima Pref.

The present paper deals with the toxicity levels in association with *Gonyaulax* bloom, of various bivalves collected in these areas during 1976~77. Some mention is also made on the toxicity of scallops collected in Ofunato Bay, Iwate Pref. where rather a frequent occurrence of toxic scallops has been known since 1961^{2,3)}.

Materials and Methods

Specimens of bivalves analyzed were collected as follows: short-necked clam *Tapes philippinarum*, "isoshijimi" *Soletellina olivacea*, "onogai" *Mya arenaria onogai*, oyster *Crassostrea gigas*, "shio-fukigai" *Macra veneriformis*, "bakagai" *Macra chinensis*, clam *Meretrix lusoria*, "mategai" *Solen strictus*, mussel *Mytilus* sp., and "uchimurasaki" *Saxidomus purpuratus* at several localities mostly along the west coast of Ise Bay and Ago Bay

(Fig. 1), mussel at several localities of Owase Bay; short-necked clam at a locality of Komatsushima Bay; scallop *Patinopecten yessoensis* and "akazara" *Chlamys nipponensis akazara* at a locality of Ofunato Bay.

The bivalves collected were immediately frozen and kept in a deep freezer at -20°C until used.

Fig. 1. Map showing localities of collection in Ise Bay.

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Table 1. Toxicity of bivalves collected at several localities in Ise Bay (MU/g)

Locality of collection	Date of collection	Short-necked clam	"Iso-shijimi"	"Ono-gai"	Oyster	"Shio-fukigai"	"Baka-gai"	Clam	"Mate-gai"	Mussel
Tsu	Apr. 4, '76	12								
	" 15, "	60			20	14				40
	" 17, "	30								
	" 23, "		8							
	" 25, "	5	18	8	< 2	< 2				13
	May 1, "	< 2	6	< 2		< 2			< 2	6
	" 9, "	< 2	18			< 2				2
	" 15, "	< 2				< 2	< 2		< 2	
	" 23, "	< 2	6							< 2
	" 29, "	< 2	3	< 2		< 2				< 2
	Jun. 12, "	< 2	< 2							
	Nov. 23, "		< 2							
	Jan. 20, '77		2							
	Mar. 11, "		3							
	Apr. 6, "						< 2			
	" 17, "		< 2							
	" 23, "	< 2	< 2					< 2		
Mikumo	Jun. 2, "	< 2	< 2		< 2	< 2	< 2	< 2		
	Oct. 13, "	< 2	< 2							
	Nov. 13, "	< 2	< 2					< 2		
	Apr. 19, '76	5								
	Apr. 15, '76	100	17	13		14	25	11	7	
	" 18, "	100	20				13	6	21	13
	" 22, "	10								
	" 25, "	5								
	May 2, "	2						2		
	" 9, "	< 2					< 2			
Matsunase	" 30, "	< 2								
	Mar. 24, '77		2							
	Apr. 24, "	< 2								
	Oct. 14, "	< 2								
	Nov. 13, "	< 2								
Ominato	Apr. 27, "	< 2								
	May 16, "	< 2								
Toba	Apr. 17, "	7								
	Apr. 24, "	< 2								
	May 4, "	< 2								
Sakate	" 16, "	< 2								
	Apr. 18, "	< 2								
Memotori	Apr. 29, "	< 2								

The extract for toxicity test was prepared from a pooled sample of several whole soft bodies of each bivalve, according to the A.O.A.C. method for paralytic shellfish poison⁴¹. With the scallop and "akazara", extracts were prepared from each of mid-gut gland, ovary, and adductor muscle. Lethality to mice was determined by the method previously applied for saxitoxin of toxic crabs⁵¹.

Results

Toxicity levels of the bivalves collected at several localities in Ise Bay are shown in Table 1. As seen in Table 1, most bivalves collected in April and May, 1976, at Tsu, Mikumo, Matsunase, and Toba exhibited high to moderate toxicity levels, while those collected at other localities near the mouth of this bay remained non-toxic. In April

of the same year, a bloom was observed over a fairly wide area mainly along the west coast of Ise Bay. *Gonyaulax catenella* variety was tentatively identified as the plankton responsible for letting the bivalves toxic. At any rate, a close association of toxic bivalves with a *Gonyaulax* plankton was thus recognized. Toxicity tests showed that "isoshijimi" specimens at Tsu and Matsunase became toxic again in 1977, though no visible bloom was observed this time.

Among the bivalves collected at Tsu, the short-necked clam and mussel exhibited the highest toxicity levels on April 15, 1976, 60 and 40 MU/g, respectively (Table 1). The toxicity level of "isoshijimi" remained at a fairly high level of 18 MU/g until early May, differing from those of other bivalves. At Matsunase, the highest toxicity level (100 MU/g) was recorded by the short-necked clam collected on April 15 and 18, 1976. Toxicity levels of other bivalves collected simultaneously were in a range of 6 to 25 MU/g.

The short-necked clam collected on May 5, 1976, in Ago Bay, and "uchimurasaki" collected in April, 1976, at Irako on the east coast of Ise Bay were both found to be non-toxic at all.

As described before¹⁾, a bloom with *Gonyaulax catenella* as the dominant species occurred in Owase Bay through January and February, 1975, infesting various bivalves there with the toxin involved. However, any *Gonyaulax* bloom was not observed in the same season of 1977. Table 2

Table 2. Toxicity of mussel specimens collected at several localities in Owase Bay (MU/g)

Date of collection	Locality of collection			
	Hikimoto	Nagahama	Ikuma	Yaguchi
Jan. 7, '77	<2	<2	<2	2
" 18, "	<2	2	2	3
" 28, "	2	<2	8	11
Feb. 8, "	2	11	19	70
" 18, "	4	16	14	24

shows the results of toxicity determination for the mussels collected then at several localities in Owase Bay. The mussel specimens collected at Yaguchi became toxic as early as January 7, raising gradually the toxicity up to a maximum score of 70 MU/g on February 8. Ikuma samples also exhibited the highest toxicity (19 MU/g) on the same day. The toxicity levels at two other localities were still increasing until February 18. These levels are generally lower than those of mussels collected in

1975¹⁾.

In June, 1976, a bloom of *Gonyaulax catenella* occurred in Komatsushima Bay. Toxicity tests were performed on the short-necked clam specimens collected there in mid-July, demonstrating them to be non-toxic.

Table 3 shows the toxicity levels of mid-gut gland, ovary, and adductor muscle of the scallop and "akazara" collected in May, 1976, in Ofunato Bay. Both kinds of bivalves were found to be fairly toxic already on May 12. On May 25, the toxicity level of mid-gut gland of the scallop was

Table 3. Toxicity of various parts of two bivalves collected in Ofunato Bay (MU/g)

	Date of collection	Mid-gut gland	Ovary	Adductor muscle
Scallop	May 12, '76	16	2	2
	" 25, "	800	45	20
"Akazara"	May 12, '76	16	2	2
	" 25, "	200	32	4

as high as 800 MU/g, followed by 45 MU/g in ovary and 20 MU/g in adductor muscle. The corresponding levels of "akazara" were 200, 32, and 4 MU/g, respectively. No visible bloom was observed in Ofunato Bay this time either.

Discussion

As described above, the occurrence of toxic mussel was observed again in Owase Bay through January to February, 1977, though no visible bloom was observed this time. *Gonyaulax*-associated-toxic bivalves appeared also along the west coast of Ise Bay in April to May, 1976. A slightly toxic bivalve occurred again there in 1977, but no bloom was recognized in this year.

On the other hand, it is well known that the scallop and "akazara" become toxic in the early summer every year in Ofunato Bay, though any responsible bloom has not so far been observed. In 1961, one of us (R. A., unpublished) found a possibly responsible dinoflagellate tentatively identified as *Gonyaulax catenella* variety in the surrounding water of toxic scallop or "akazara": Its plate pattern (4', Oa, 6'', 6''', 1p, and 1''') was quite indistinguishable from that of *Gonyaulax catenella*, while the well-developed curtain fin specific to the latter dinoflagellate was absent in the Ofunato plankton. The same dinoflagellate may be associated again with the occurrence of both toxic bivalves in 1976, though it is still premature

to draw any conclusion on this matter as yet.

The maximum toxicity level encountered this time was as high as 800 MU/g with the mid-gut gland of scallop. Another high toxicity level of 200 MU/g was recorded with the mid-gut gland of "akazara". No visible bloom was observed this time either. This reminds us the comparable cases in Fundy Bay⁶¹ or at the estuary of St. Lawrence River⁶¹.

Toxic bivalves occurred also in Ise Bay through April to May, 1976, with the highest toxicity score being 100 MU/g in the short-necked clam (Table 1). It is generally accepted that the lethal dose of paralytic shellfish poison is 3,000~5,000 MU, depending on physical conditions, in human⁷¹. These lethal doses are rather easily attained by toxic bivalves: *e.g.*, 30~50 g of the whole soft part of short-necked clam with the toxicity at 100 MU/g, or 150~250 g of the scallop adductor muscle with the toxicity of 20 MU/g. Hence, toxic bivalves as encountered this time seem extremely hazardous from the viewpoint of public health. Incidentally, it should be noted that bivalves become toxic in Ise Bay through April to May, the best season for picnicking: some people may collect toxic bivalves at beaches or eat them, resulting in poisoning.

It has been well known in Canada and U.S.A. that bivalves become toxic almost every year and in almost the same season in the once-affected area. Our present data seem to agree with this. As described above, toxic bivalves occur with or without a prelude of the *Gonyaulax* bloom. Therefore, it is quite necessary to establish a monitoring system in each affected area, in order to survey periodically the occurrence of *Gonyaulax* bloom and/or toxic bivalves. In this connection, even in the same locality and season, bivalves become toxic in their respective ways, supposedly reflecting

their differences in catabolizing or excreting ability of the toxin(s) ingested (Tables 1 and 3). It is reasonable, therefore, to select as "index bivalve" the most easily affected ones: *e.g.*, the short-necked clam or mussel at Tsu and the short-necked clam at Matsunase.

The purification and characterization of the toxins involved, as well as the final identification of Ofunato dinoflagellate, are now in progress.

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